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**APPLICATION NUMBER: 60/485,503**

**FILING DATE: *July 08, 2003***

**RELATED PCT APPLICATION NUMBER: *PCT/US04/22165***

Certified by



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Attorney Docket No. P35892  
Express Mail Label No. EV 342493242US

15015 U.S. PTO  
60/485503  
07/08/03

## PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)					
Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)			
Bertram	Jacobs	Tempe, Arizona			
Chandra	Mitnik	Tempe, Arizona			
Langland	Jeffrey	Chandler, Arizona			
<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
<b>TITLE OF THE INVENTION (280 characters max)</b>					
MUTANTS OF VACCINIA VIRUS AS ONCOLYTIC AGENTS					
<b>Direct all correspondence to: CORRESPONDENCE ADDRESS</b>					
<input checked="" type="checkbox"/> Customer Number		21003		Place Customer Number Bar Code Label here	
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<b>ENCLOSED APPLICATION PARTS (check all that apply)</b>					
<input checked="" type="checkbox"/> Specification Number of Pages		10		<input type="checkbox"/> CD(s), Number	
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets		11		<input checked="" type="checkbox"/> Other (specify)	
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76		1 PAGE aBSTRACT			
<b>METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT</b>					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.		<input type="checkbox"/> A check or money order is enclosed to cover the filing fees		FILING FEE AMOUNT (\$)	
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<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input type="checkbox"/> No.					
<input checked="" type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are:					
Wallace Foundation TDT8786					

Respectfully submitted,

SIGNATURE

*Carmella L. Stephens*

TYPED or PRINTED NAME

Carmella L. Stephens

TELEPHONE

212 408-2539

Date: July 8, 2003

REGISTRATION NO.

(if appropriate)

Docket Number:

41,328

P35892

**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**



# FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$)**80**

## Complete If Known

Application Number	
Filing Date	July 8, 2003
First Named Inventor	Jacobs et al.
Examiner Name	
Art Unit	
Attorney Docket No.	P35892

## METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☒ Deposit Account:

Deposit Account Number: **02-4377**  
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### 1. BASIC FILING FEE

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1001 750	2001 375	Utility filing fee	
1002 330	2002 165	Design filing fee	
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SUBTOTAL (1)			(\$) <b>80</b>

### 2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims  - 20 =  0 x  =  0  
Independent Claims  - 3 =  0 x  =  0  
Multiple Dependent  =

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description
1202 18	2202 9	Claims in excess of 20
1201 84	2201 42	Independent claims in excess of 3
1203 280	2203 140	Multiple dependent claim, if not paid
1204 84	2204 42	** Reissue independent claims over original patent
1205 18	2205 9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) (\$)**0**

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1051 130	2051 65	Surcharge - late filing fee or oath	
1052 50	2052 25	Surcharge - late provisional filing fee or cover sheet	
1053 130	1053 130	Non-English specification	
1812 2,520	1812 2,520	For filing a request for <i>ex parte</i> reexamination	
1804 920*	1804 920*	Requesting publication of SIR prior to Examiner action	
1805 1,840*	1805 1,840*	Requesting publication of SIR after Examiner action	
1251 110	2251 55	Extension for reply within first month	
1252 410	2252 205	Extension for reply within second month	
1253 930	2253 465	Extension for reply within third month	
1254 1,450	2254 725	Extension for reply within fourth month	
1255 1,970	2255 985	Extension for reply within fifth month	
1401 320	2401 160	Notice of Appeal	
1402 320	2402 160	Filing a brief in support of an appeal	
1403 280	2403 140	Request for oral hearing	
1451 1,510	1451 1,510	Petition to institute a public use proceeding	
1452 110	2452 55	Petition to revive - unavoidable	
1453 1,300	2453 650	Petition to revive - unintentional	
1501 1,300	2501 650	Utility issue fee (or reissue)	
1502 470	2502 235	Design issue fee	
1503 630	2503 315	Plant issue fee	
1460 130	1460 130	Petitions to the Commissioner	
1807 50	1807 50	Processing fee under 37 CFR 1.17(q)	
1806 180	1806 180	Submission of Information Disclosure Stmt	
8021 40	8021 40	Recording each patent assignment per property (times number of properties)	
1809 750	2809 375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810 750	2810 375	For each additional invention to be examined (37 CFR 1.129(b))	
1801 750	2801 375	Request for Continued Examination (RCE)	
1802 900	1802 900	Request for expedited examination of a design application	

Other fee (specify)

\*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$)**0**

## SUBMITTED BY

Name (Print/Type)	<b>Carmella L. Stephens</b>	Registration No. (Attorney/Agent)	<b>41,328</b>	Telephone	<b>212 408-2539</b>
Signature	<i>Carmella L. Stephens</i>	Date	<b>July 8, 2003</b>		

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TO ALL WHOM IT MAY CONCERN:

Be it known that WE, Bertram Jacobs, Chandra Mitnik, and Jeffrey Langland, a citizens of the United States, whose post office addresses are, 1004 S. Wilson Street, Tempe, Arizona 85281, 1056 E. Sandpiper Drive, Tempe, Arizona 85283, and 506 W. El Alba Way, Chandler, Arizona 85225, respectively, have invented an improvement in:

MUTANTS OF VACCINIA VIRUS AS ONCOLYTIC AGENTS

of which the following is a

SPECIFICATION

INTRODUCTION

The present invention relates to mutant oncolytic vaccinia viruses and their use for selective destruction of cancer cells. The mutant vaccinia viruses of the invention include those having a reduced ability to inhibit the antiviral dsRNA-dependent protein kinase (PKR). Such mutants include, for example, vaccinia viruses having mutations in the E3L region. The invention is based on the discovery that vaccinia viruses having mutations in the E3L region are capable of replication in oncogenic *ras* expressing cells resulting in cell lysis. The invention further provides methods for treating *ras*-mediated proliferative disorders, such as neoplasms, in a host comprising administration of mutant vaccinia virus under conditions which result in substantial lysis of the proliferating cancer cells.

### BACKGROUND OF INVENTION

Most current cancer treatments have some selectivity for cells that divide rapidly, such as cancer cells, intestinal cells, and hair follicle cells, but ultimately fail to take advantage of the molecular differences between tumor and normal cells. Oncolytic ("onco" meaning cancer, "lytic" meaning killing) viruses represent a promising new cancer therapy that seeks to exploit the natural properties of viruses to aid in the fight against cancer. Oncolytic viruses are viruses that infect and replicate in cancer cells, destroying the cancer cells and leaving normal cells largely unaffected. Such viruses include reoviruses (Wilcox et al., 2001, J. Natl. Cancer Inst. 93:903-912; Coffey et al., 1998, Science 2:83:1332-1331; Norman et al., 2002, Human Gene Therapy 13:641-642; Strong et al., 1998, 12:3351-3362), vesicular stomatitis virus (VSV) (Stojdl, 2000 nature 6:821-825), herpes simplex virus (HSV) (Farasetti et al., Nature Cell Biology 3:745) and human influenza A virus (Bergmann et al., 2001 Cncr Research 64:8188-8193).

The *ras* protein plays a central role in a variety of cellular processes in vertebrates and invertebrates. Active *ras*, through a kinase cascade, is responsible for cell differentiation and proliferation in response to normal mitogenic signals. A mutation in the *ras* gene can cause uncontrolled cell growth, leading to tumor formation. It has been demonstrated that a large number of tumors contain a mutated *ras* gene that results in a constitutively expressed or always active form of *ras*, thus proving to be an effective genetic marker of tumor cells and a potential attractive target for therapy.

In addition to these cell growth activities, the *ras* pathway alters the anti-viral interferon pathway. The interferon system acts as an alarm for the host by warning nearby cells



of an impending virus attack. After a cell receives the warning signal of interferon, a biochemical cascade is activated resulting in the induction of hundreds of genes. Among these genes induced by interferon, is the well-studied antiviral dsRNA-dependent protein kinase (PKR). This enzyme becomes activated in the presence of the double-stranded RNA produced during most viral infections. The activated PKR inhibits protein synthesis in order to halt the viral infection. The *ras* pathway results in an increase in an inhibitor of PKR, which effectively blocks this step in the interferon pathway. This inhibitor has been termed RIKI, which stands for *ras*-inducible PKR kinase inhibitor. RIKI is believed to be associated with a weak tyrosine or serine/threonine phosphatase activity. Thus, it disables PKR by dephosphorylation, leading to an inactive form of PKR.

Many viruses, including vaccinia virus, have developed mechanisms in order to evade the host defense system, specifically the actions of interferon. One of the ways that vaccinia virus subverts the host immune response is by encoding the protein E3L. As noted above, PKR becomes activated by the double-stranded RNA produced during viral infections. Vaccinia virus masks the double-stranded RNA it produces with the E3L protein, which binds and sequesters double-stranded RNA. The masked double-stranded RNA cannot activate PKR. Consequently, viral protein synthesis continues even in the presence of interferon, and the viral infection proceeds unimpeded.

Use of vaccinia virus as an oncolytic agent offers several advantages over other oncolytic viruses. First, the viruses can be genetically engineered with ease. Thus, by inserting or deleting genes from vaccinia, the safety and efficacy of the virus can be enhanced. An additional advantage is the wide base of knowledge concerning vaccinia virus infections in

humans. Finally, vaccinia virus has been shown to be safe in all but immunocompromised individuals.

By creating various deletion mutants in the vaccinia virus E3L region, viruses have been created that are lacking in their ability to inhibit PKR and, thus, become dependent on the PKR inhibitory activity found in *ras*-transformed cells. Such viruses provide a means for targeting selective cell lysis to *ras* transformed cells.

#### SUMMARY OF THE INVENTION

The present invention relates to mutant oncolytic vaccinia viruses and the use of such viruses for selective destruction of cancer cells. The mutant vaccinia viruses of the invention include those having a reduced ability to inhibit the antiviral dsRNA-dependent protein kinase (PKR). Such mutants include, for example, vaccinia viruses having mutations in the E3L region.

The invention is based on the discovery that vaccinia viruses having mutations in the E3L region are able to replicate in oncogenic *ras* expressing cells resulting in cell lysis. As demonstrated herein, several mutant vaccinia viruses are shown to be oncolytic with specificity for a particular molecular pathway that is commonly dysregulated in a variety of cancers. These vaccinia viruses are dependent on the overexpression of *ras*, a key molecular characteristic of over 50% of cancers. Thus, the present invention provides methods for treating *ras*-mediated proliferative disorders in a host wherein said method comprises administration of mutant vaccinia virus under conditions which result in substantial lysis of proliferating cancer cells.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Deletion mutants of E3L in vaccinia virus and their PKR inhibitory and *ras* dependency characteristics.

Figure 2. NIH-3T3 or NIH-3T3 overexpressing the *ras* protein were either mock infected or infected with the above identified vaccinia virus constructs at an MOI (multiplicity of infection) of 0.01. Cytopathic effect is a description of any adverse properties of cells following infection. Photographs were taken at 24, 48 and 72 hours post infection to record cytopathic effect. In Figure 2A, all cells were mock infected and appear normal and healthy through 72 hours post infection. In Figure 2B, cells were infected with wt WR virus, which is not *ras* dependent. Cytopathic effect was noted in both the NIH-3T3 and NIH-3T3 Ha-Ras beginning at 48 hours post infection and continuing to 72 hours post infection. In Figure 2B, cells were infected with wt WR virus, which was not *ras* -dependent. Cytopathic effect was noted in both NIH-3T3 and NIH-3T3 Ha-Ras beginning at 48 hours post infection and continuing to 72 hours post infection. Slight cytopathic effect was noted in Figure 2E, when cells were infected with WRdel7C, indicating that this virus is less *ras*-dependent than the other mutant viruses. Cytopathic effect was not evident in Figures 2C, 2D and 2F in the NIH-3T3 cells, indicating that these virus constructs are *ras*-dependent.

Figure 3. This figure represents viral replication over a 72-hour period. NIH-3T3 or NIH-3T3 Ha-Ras cells were infected with wtWR, WRdel183N, WRdel54N, and WRdelE3L at an MOI of 0.01. Viral replication was measured by determining how many infectious virus particles were present after 72 hours. The number of infectious virus particles is expressed as

titer and is on the y-axis, while the various vaccinia constructs are depicted on the x-axis.

WtWR grew to high titers in both cell lines. Titers dropped in the NIH-3T3 cells, but remained high in the NIH-3T3 Ha-Ras cells for all of the vaccinia constructs.

Figure 4. This figure represents viral replication over a 72-hour period. Either normal breast cells or cancerous breast cells were infected with wtWR, WRde154N, and WRde1E3L at an MOI of 0.01. Viral replication was measured by determining how many infectious virus particles were present after 72 hours. The number of infectious virus particles is expressed as titer and is on the y-axis, while the various vaccinia constructs are depicted on the x-axis. WtWR grew to high titers in all cell lines. WRde1E3L failed to grow in any cell line. WRde154N did not grow in the normal breast cells, or in two of the cancer cell lines. However, WRde154N grew to high titers in four out of six breast cancer cell lines.

Figure 5. This figure depicts viral replication by measuring protein synthesis. NIH-3T3 or NIH-3T3 Ha-Ras cells were either mock infected or infected with wtWR, WRde183N, WRde154N, WRde126C, or Wrde1E3L. At 72 hours post infection, the cells were harvested and their proteins loaded onto this gel. This gel was then probed with antibodies against vaccinia virus in order to detect vaccinia virus proteins. Vaccinia virus proteins were not detected in either mock infection. Vaccinia virus proteins were detected in wtWR and less in WRde183N infected NIH-3T3 cells. Viral protein synthesis was not detected in WRde154N, WRde126C, or Wrde1E3L infected NIH-3T3 cells. Viral protein synthesis was detected in all infected NIH-3T3 Ha-Ras cells, with lower levels noted in WRde154N infected cells.

Figure 6. To confirm the endogenous inhibition of PKR in *ras*-transformed cells, a PKR phosphorylation assay was conducted. NIH-3T3 or NIH-3T3 Ha-Ras cells were either incubated with interferon to induce production of PKR or were not incubated. The cells were harvested and subjected to an *in vitro* kinase assay. Briefly, cell lysates were incubated with or without double-stranded RNA to activate the PKR and radioactively labeled substrate to detect the phosphorylation event (representing PKR activation). These lysates were purified and loaded onto a gel, that was subsequently exposed to x-ray film to detect any radioactive PKR. The intensity of each PKR band was measured using the computer software ImageQuant, and the relative intensities were graphed. High levels of activated PKR were detected in NIH-3T3 cells that were incubated with both double-stranded RNA and interferon. The effect was dampened in the NIH-3T3 Ha-Ras cells.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to mutant oncolytic vaccinia viruses and the use of such viruses for selective destruction of cancer cells. The mutant vaccinia viruses of the invention comprise mutant vaccinia viruses with a reduced ability to inhibit the antiviral dsRNA-dependent protein kinase (PKR). In particular, the present invention provides recombinant vaccinia virus from which the region encoding the E3L gene product has been inactivated. Such inactivation may result from partial or complete deletion of the E3L region or, alternatively, substitution of nucleotides within the E3L region that result in inactivation of the E3L gene product. The invention is based on the discovery that such mutant viruses are unable to inhibit PKR thus rendering the viruses dependent on the PKR inhibitory activity found in *ras*-transformed cells.

The E3L gene product of the vaccinia virus is a 190 amino acid polypeptide. The E3L gene codes for several functions including a dsRNA-binding protein, a Z-DNA-binding protein, and dimerization. Amino acids 118-190 have been implicated in dsRNA binding, as disclosed by Chang and Jacobs (1993, *Virology* 194:537-547). Amino acid numbering as used herein is adopted from Goebel et al., 1990, *Virology* 179:247-66, 577-63.

According to the invention "deletion of the E3L gene" and its grammatical equivalents refer to a vaccinia virus wherein a nucleic acid encoding all 190 amino acids or a subset of the 190 amino acids of E3L are not present. According to the invention, if the vaccinia virus having a deletion in the E3L gene has a residual nucleic acid encoding a subset of the 190 amino acids of E3L, said residual nucleic acid is incapable of producing a functional gene product or the gene product is incapable of binding dsRNA. The ability of the E3L gene product to bind to dsRNA can be determined by binding assays known in the art and disclosed, for example, by Chang et al., 1993, *Virology* 194:537.

Deletion of the E3L gene from vaccinia virus results in a virus that is interferon-sensitive, but also is highly debilitated for replication in many cells in culture (Jacobs and Langland, 1996, *Virology* 219(2):339-349). However, as demonstrated herein, such viruses are capable of replication in *ras*-transformed cells thereby providing a method for targeted cell lysis of *ras*-transformed cells.

The recombinant vaccinia virus of the present invention may be constructed by methods known in the art, and preferably by homologous recombination. Standard homologous recombination techniques utilize transfection with DNA fragments or plasmids containing

sequences homologous to viral DNA, and infection with wild-type or recombinant vaccinia virus, to achieve recombination in infected cells. Conventional marker rescue techniques may be used to identify recombinant vaccinia virus. Representative methods for production of recombinant vaccinia virus by homologous recombination are disclosed by Piccini et al., 1987, *Methods in Enzymology* 153:545.

[0001] For example, the recombinant vaccinia virus of a preferred embodiment of the present invention may be constructed by infecting host cells with vaccinia virus from which the E3L gene has been deleted. The vaccinia virus used for preparing the recombinant vaccinia virus of the invention may be a naturally occurring or engineered strain. Strains useful as human and veterinary vaccines are particularly preferred and are well-known and commercially available. Such strains include Wyeth, Lister, WR, and engineered deletion mutants of Copenhagen such as those disclosed in U.S. Patent 5,762,938. Recombination plasmids may be made by standard methods known in the art. The nucleic acid sequences of the vaccinia virus E3L gene and the left and right flanking arms are well-known in the art, and may be found for example, in Earl et al., 1993, in *Genetic Maps: locus maps of complex genomes*, O'Brien, ed., Cold Spring Harbor Laboratory Press, 1:157 and Goebel et al., 1990, *supra*. The amino acid numbering used herein is adopted from Goebel et al., 1990, *supra*. The vaccinia virus used for recombination may further comprise other deletions, inactivations, or exogenous DNA.

[0002] The present invention further provides compositions for use in targeted cell lysis wherein said compositions comprise a recombinant vaccinia virus, or viral vector, and a carrier. The term carrier as used herein includes any and all solvents, diluents, dispersion media,

antibacterial and antifungal agents, microcapsules, liposomes, cationic lipid carriers, isotonic and absorption delaying agents, and the like. Suitable carriers are known to those of skill in the art.

The compositions of the invention can be prepared in liquid forms, lyophilized forms or aerosolized forms. Other optional components, e.g., stabilizers, buffers, preservatives, flavorings, excipients and the like, can be added.

Also included in the invention is a method of treating a host with cancer, including but not limited to mammals such as a humans, with the novel compositions of the invention under conditions which result in substantial lysis of the proliferating cancer cells. In the method of the invention, the recombinant vaccinia viruses of the invention are administered to *ras*-mediated transformed cells in the host. The compositions, including one or more of the recombinant vaccinia viruses described herein, are administered using routes typically used for such administration, *i.e.*, intravenously, intravascularly, injection at site of tumor, in a suitable dose. The dosage regimen involved in the method of treating, including the timing, number and amounts of treatments, will be determined considering various hosts factors, *e.g.*, the age of the patients, time of administration and type and severity of the cancer.

Various publications are cited herein, the contents of which are hereby incorporated by reference in their entireties herein.



ABSTRACT

The present invention relates to mutant oncolytic vaccinia viruses and their use for selective destruction of cancer cells. The mutant vaccinia viruses of the invention include those having a reduced ability to inhibit the antiviral dsRNA-dependent protein kinase (PKR). Such mutants include, for example, vaccinia viruses having mutations in the E3L region. The invention is based on the discovery that vaccinia viruses having mutations in the E3L region are capable of replication in oncogenic *ras* expressing cells resulting in cell lysis. The invention further provides methods for treating *ras*-mediated proliferative disorders, such as neoplasms, in a host comprising administration of mutant vaccinia virus under conditions which result in substantial lysis of the proliferating cancer cells.

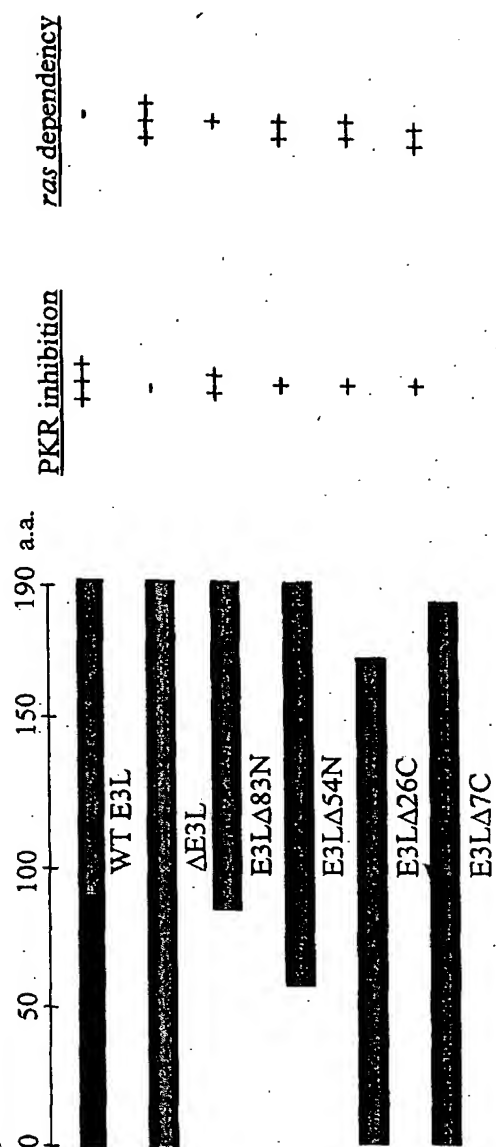


Figure 1. Deletion mutants of E3L in vaccinia virus and their PKR inhibitory and *ras* dependency characteristics.

**Figure 2a: Mock infected**

**NIH-3T3**

**NIH-3T3 Ha-Ras**

**24 HPI**

**48 HPI**

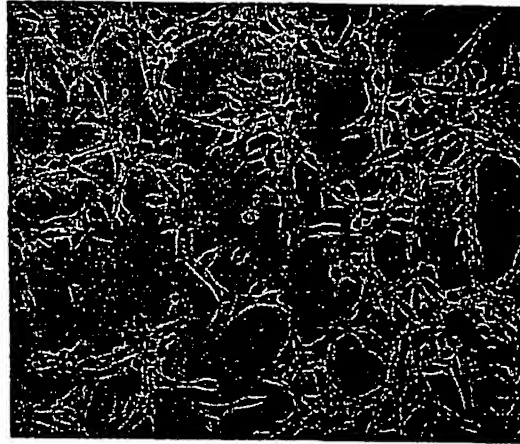
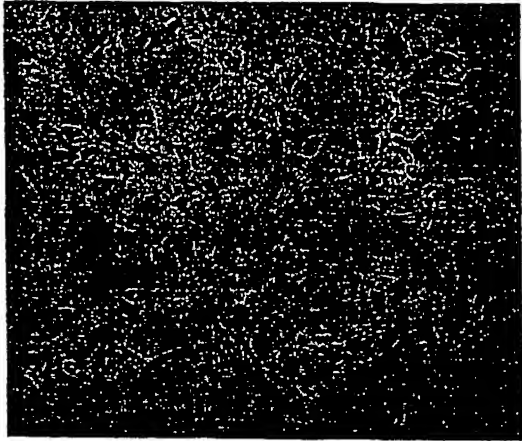
**72 HPI**

**BEST AVAILABLE COPY**

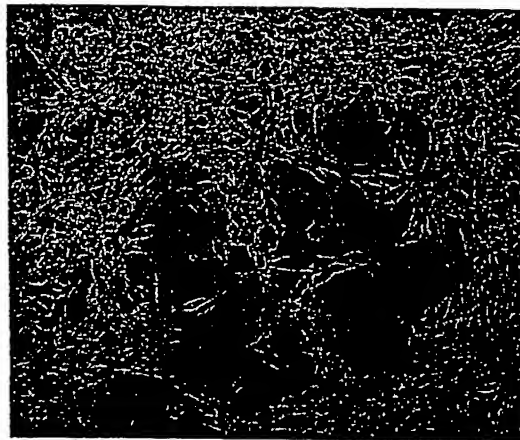
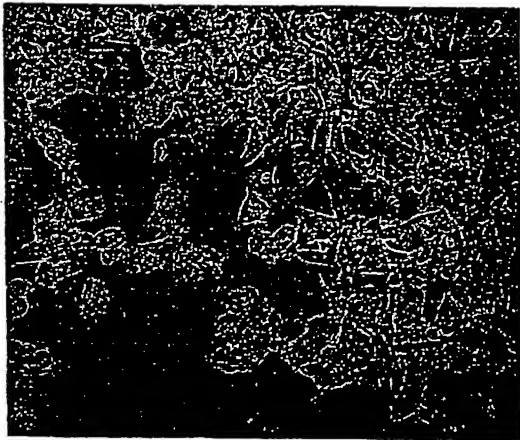
**Figure 2b: wtWR**

**NIH-3T3**

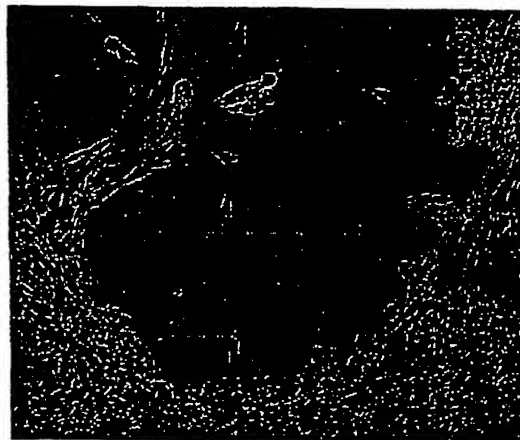
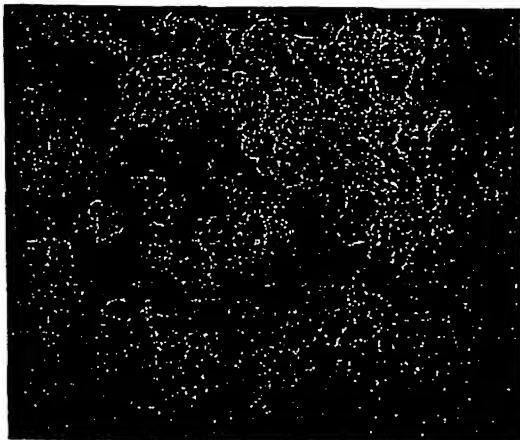
**NIH-3T3 Ha-Ras**



**24 HPI**



**48 HPI**



**72 HPI**

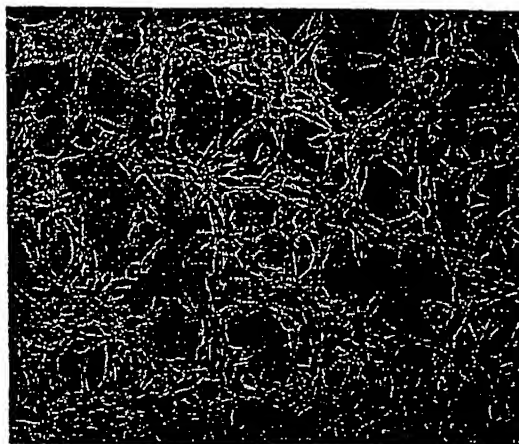
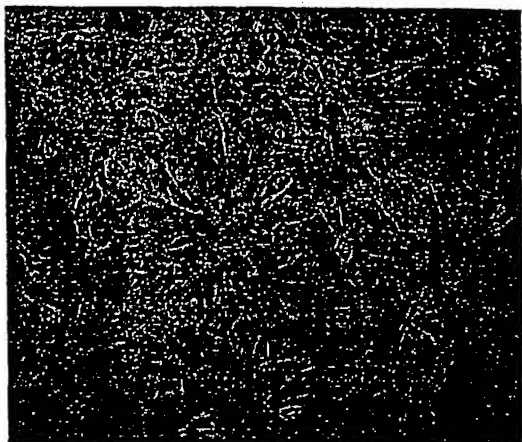
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**Figure 2c: WRdel83N**

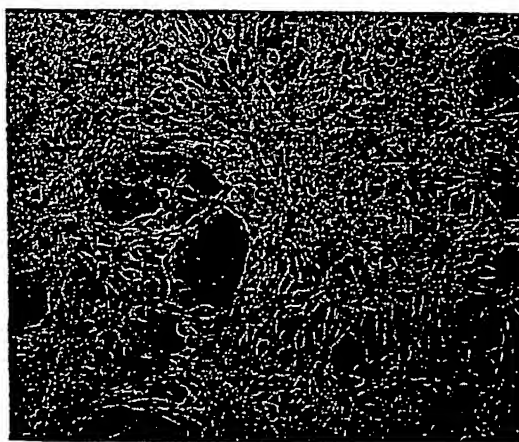
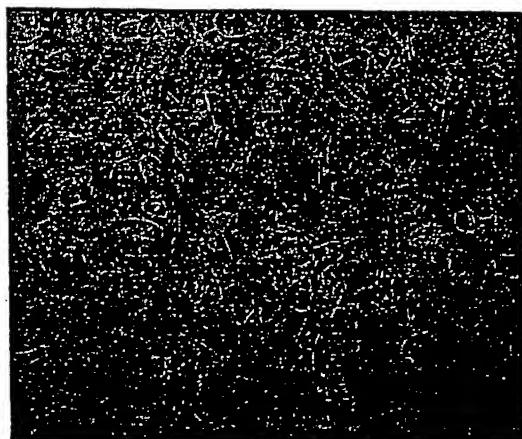
**NIH-3T3**

**NIH-3T3 Ha-Ras**

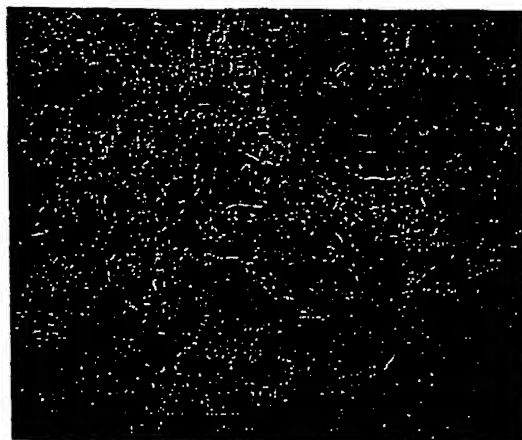
**24 HPI**



**48 HPI**



**72 HPI**

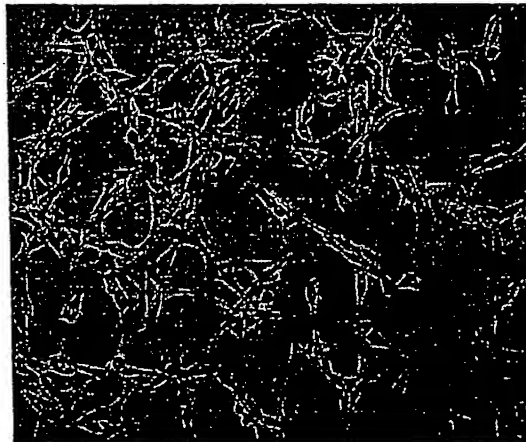
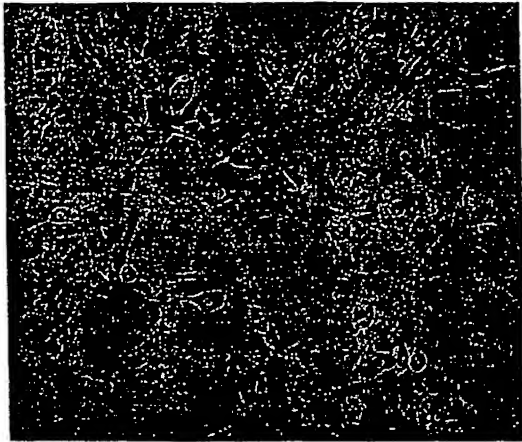


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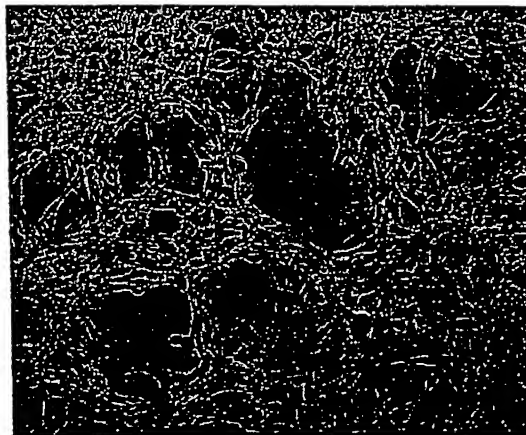
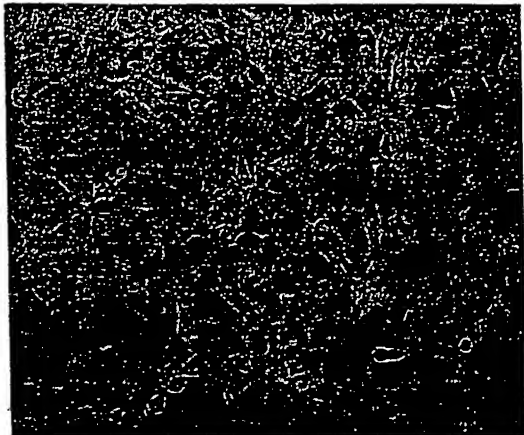
**Figure 2d: WRdel54N**

**NIH-3T3**

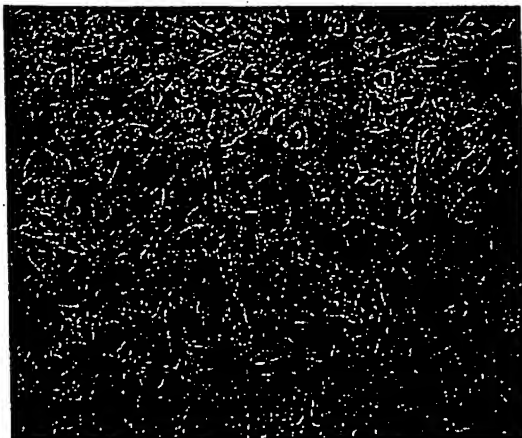
**NIH-3T3 Ha-Ras**



**24 HPI**



**48 HPI**



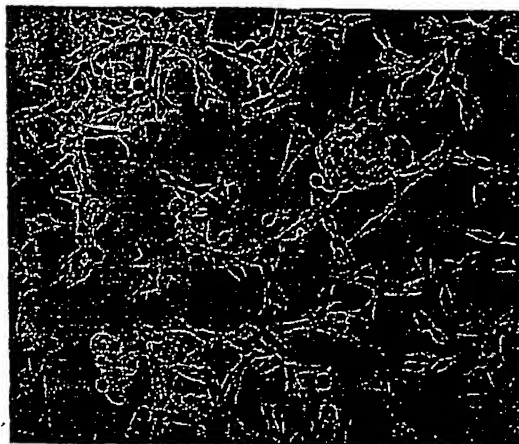
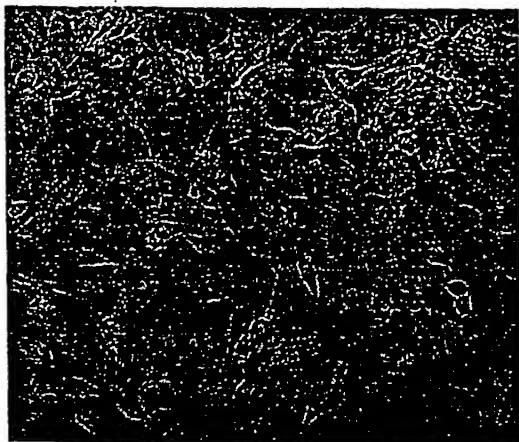
**72 HPI**

**BEST AVAILABLE COPY**

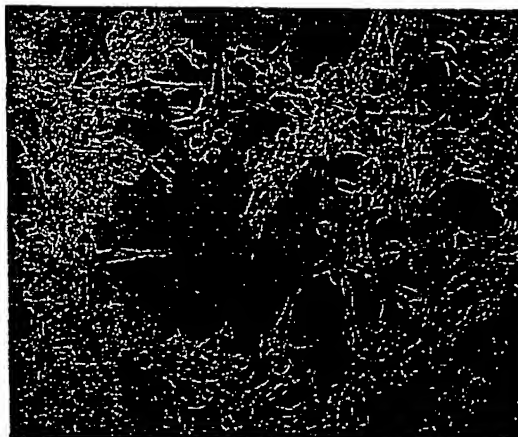
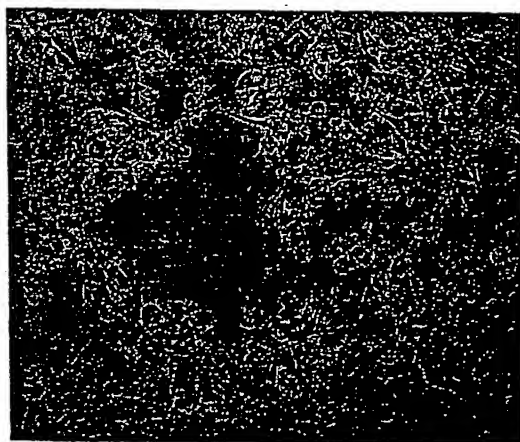
**Figure 2e: WRdel7C**

**NIH-3T3**

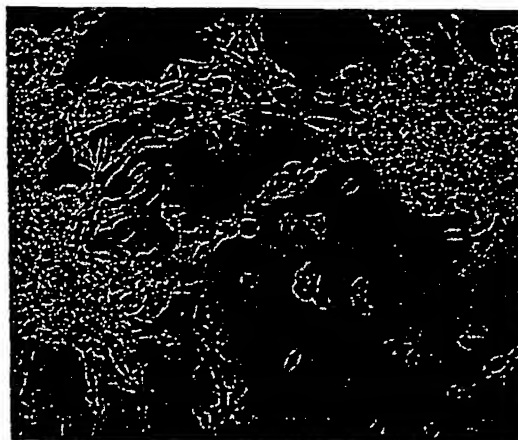
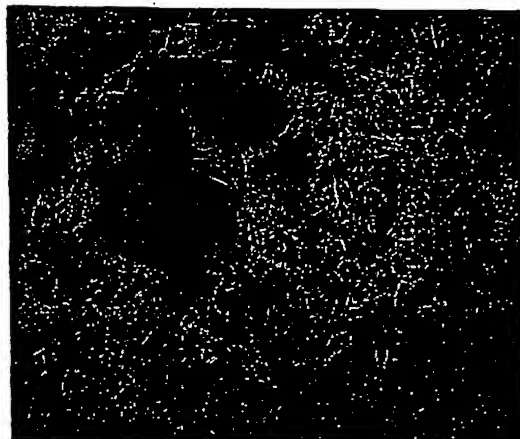
**NIH-3T3 Ha-Ras**



**24 HPI**



**48 HPI**



**72 HPI**

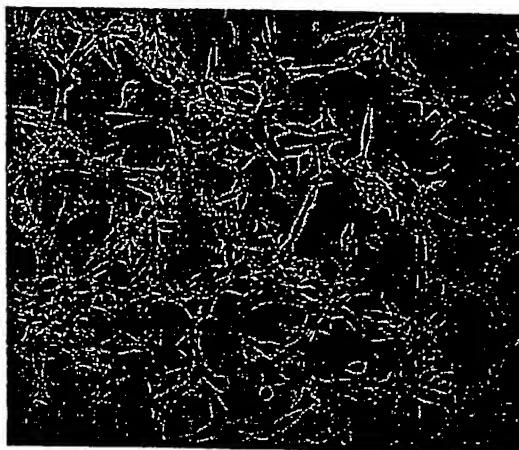
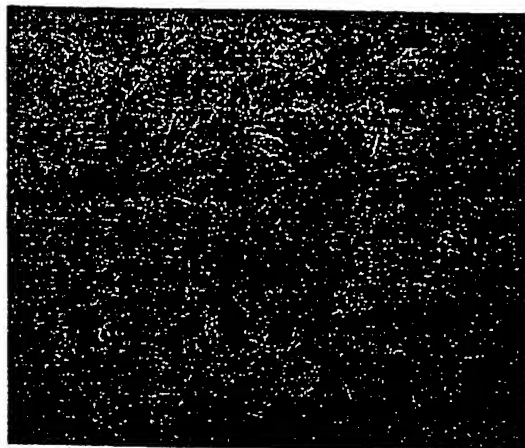
**BEST AVAILABLE COPY**



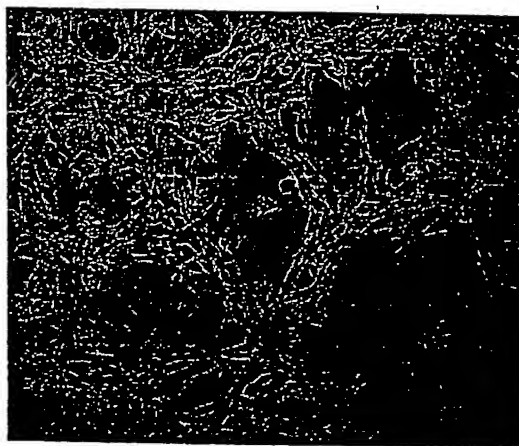
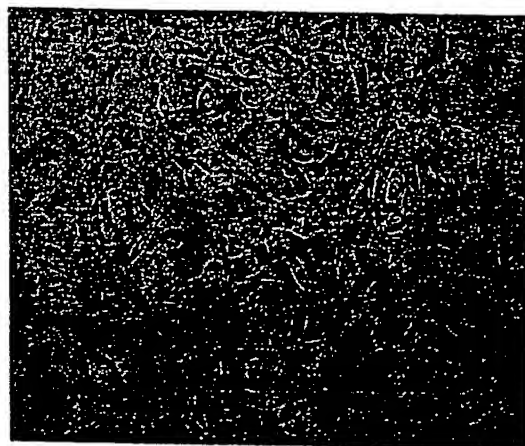
**Figure 2f: WRdelE3L**

**NIH-3T3**

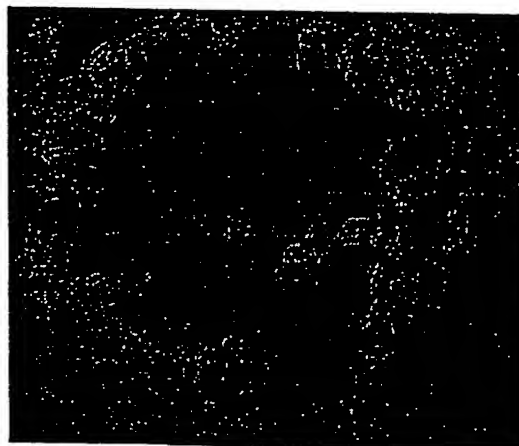
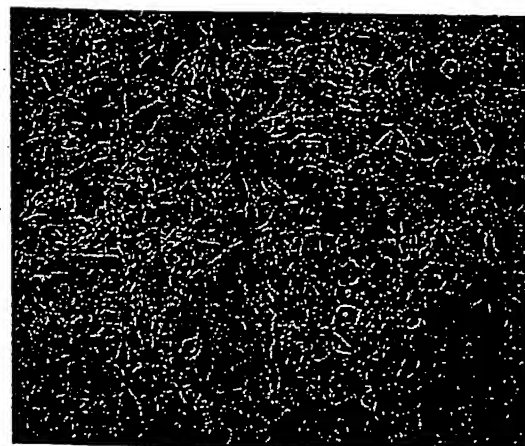
**NIH-3T3 Ha-Ras**



**24 HPI**



**48 HPI**



**72 HPI**

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Figure 3: Multi-Step Growth Curve

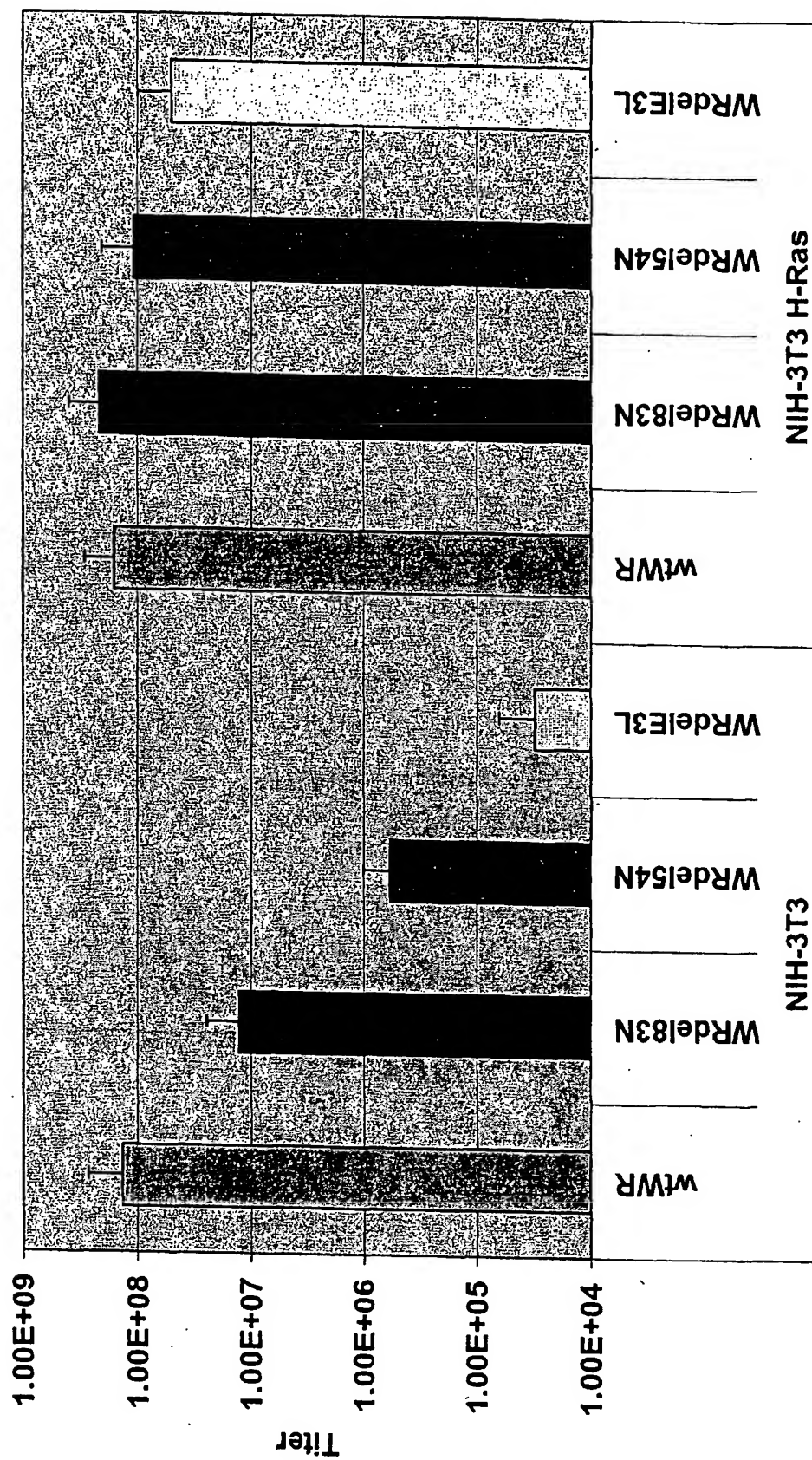
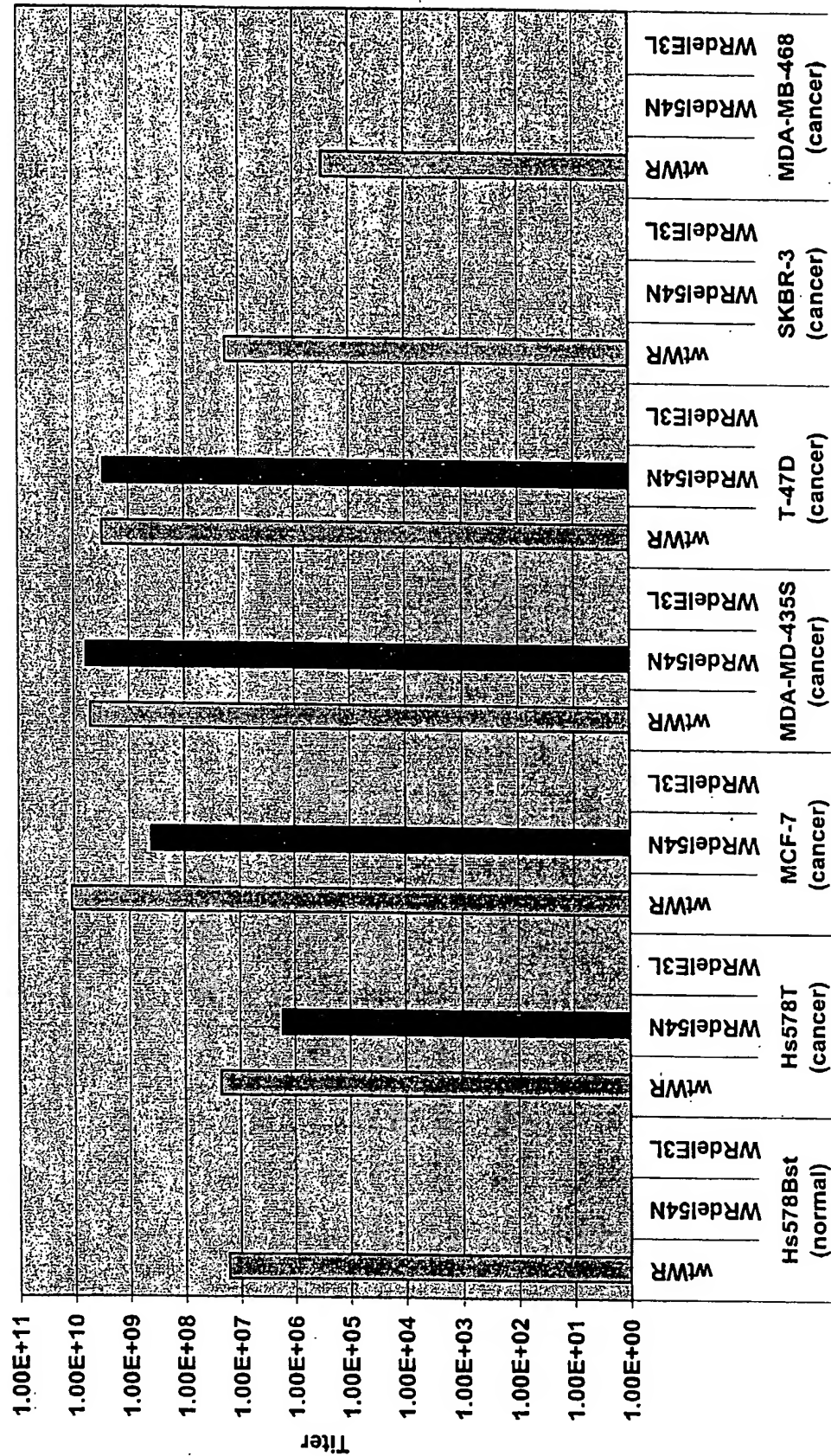


Figure 4: Multi-Step Growth Curve



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# NIH-3T3

Mock  
wtWR  
WRdel183N  
WRdel154N  
WRdel126C  
WRdel1E3L

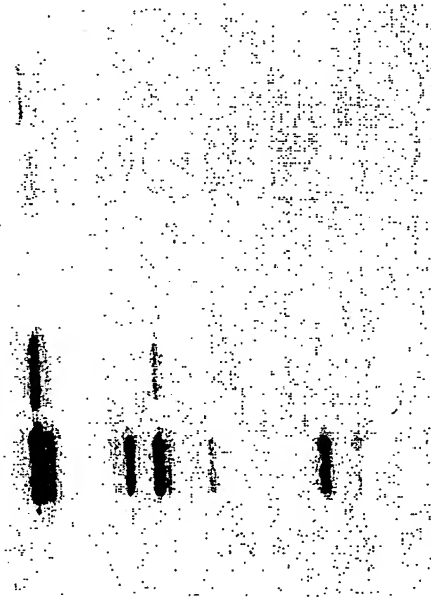


Figure 5a

# NIH-3T3 Ha-Ras

Mock  
wtWR  
WRdel183N  
WRdel154N  
WRdel126C  
WRdel1E3L

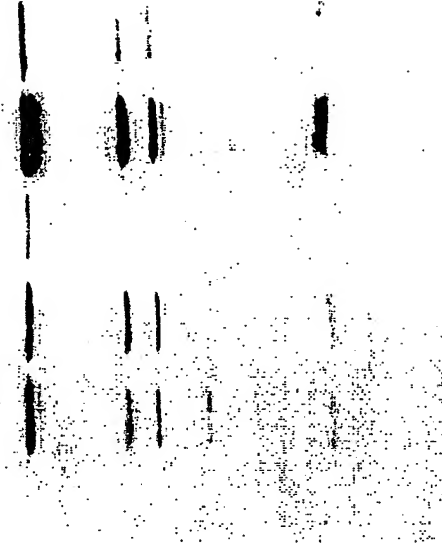
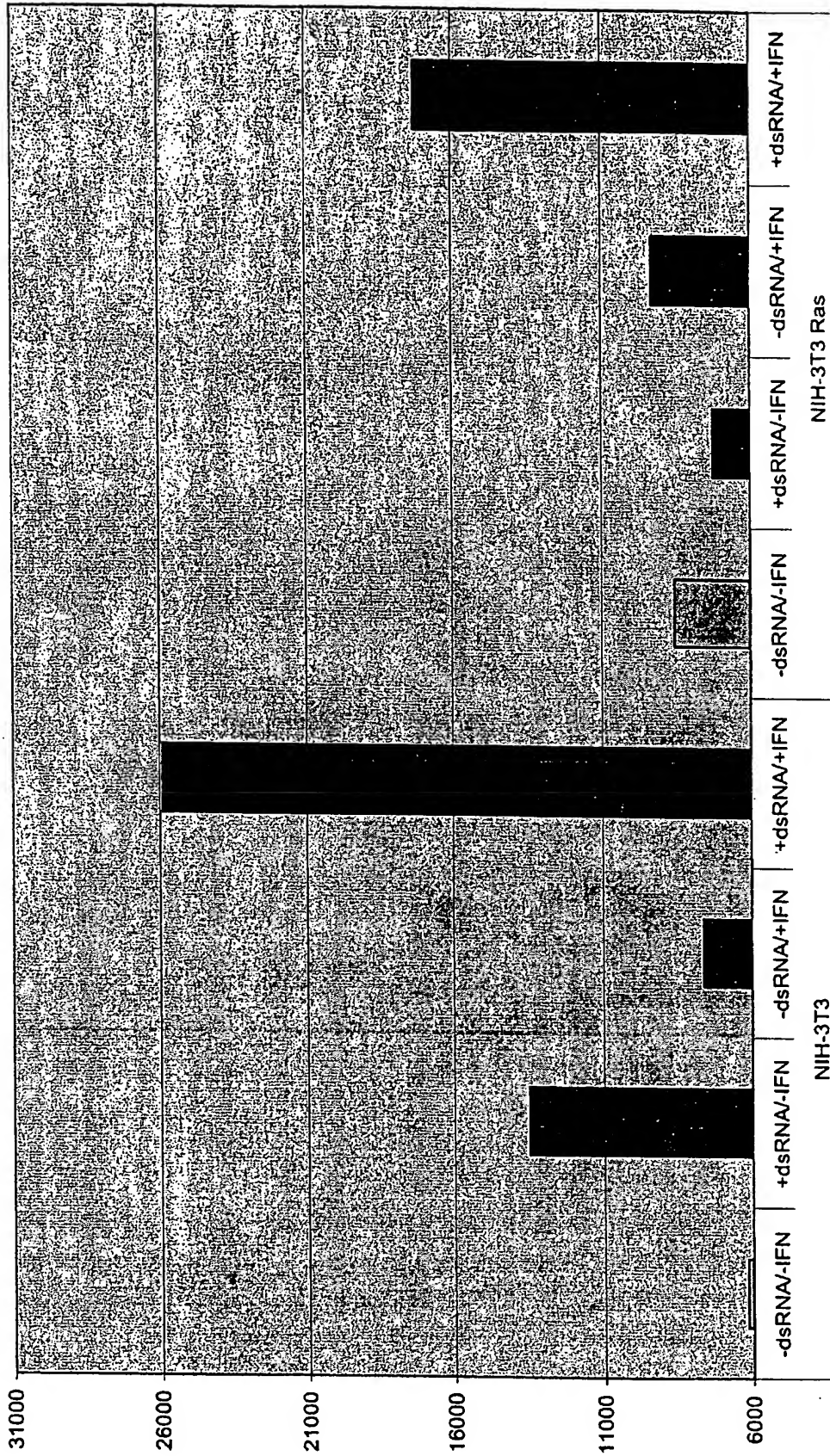


Figure 5b

Figure 6: PKR-P Assay



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